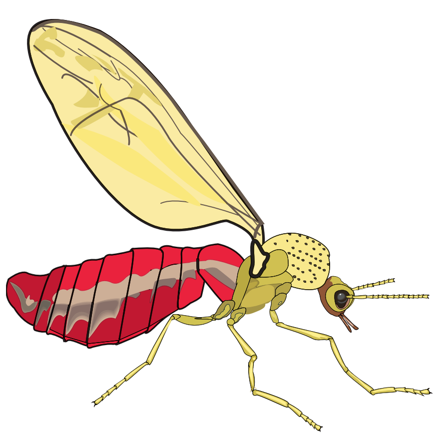
Making the Case Against the Biting Midge

Evidence is building that the biting midge, *Culicoides sonorensis,* is a vector of vesicular stomatitis virus (VSV), which causes significant economic losses in cattle, horses, and swine.



In the western United States, blood-sucking members of the fly family lay their eggs in the soft mud near water troughs that quench thirsty livestock. These tiny but hardy insects are biting midges known as *Culicoides* (ku-li-COY-deez) *sonorensis*. Adult midges are only about as long as a nickel is thick.

But size can be deceptive. *C. sonorensis* are known to be disease carriers, or vectors, that transmit bluetongue and epizootic hemorrhagic viruses among livestock and wildlife. They are also suspected vectors of other viruses, such as vesicular stomatitis virus (VSV), which infects cattle, horses, and swine.

VSV causes significant economic losses to the livestock industry from sickness, quarantines, and export limitations. Scientists believe that VSV spreads between quarantined herds by insects, but the main insect vector of VSV in the U.S. western plains is still unknown.

Microbiologist Barbara Drolet, with the ARS Arthropod-Borne Animal Diseases Research Laboratory in Laramie, Wyoming, has been studying the spread of arboviruses, such as VSV, within their insect vectors. She has been systematically building evidence to prove that *Culicoides* is a vector for VSV. "To control VSV disease outbreaks, we have to understand which insect populations transmit the disease," says Drolet.

To verify the midge as a VSV vector, Drolet first had to show that virus ingested in a blood meal infected the insect's midgut, survived, escaped from the midgut, and amplified, or replicated. She and colleagues now have proof that VSV is capable of surviving and spreading throughout *Culicoides*.

Using an artificial feeding system inside a BioSafety Level 3 laboratory, Drolet fed test midges a viral meal. To follow viral infection, she used immunohistochemistry—a process by which VSV antibodies, which bind to the virus, are detected through a series of chemical reactions. A resulting red stain, seen under a microscope, reveals the virus in the tissues.

Drolet also used in situ DNA hybridization—a technique which verifies that the virus is able to replicate itself within the insect. She showed that VSV multiplied in many tissues as it spread within the midge.

After analyzing more than 1,600 whole-body sections of 144 insects, Drolet found 3 distinct pathways of VSV infection in the midges: digestive, circulatory, and neural.

Proving that VSV can make it into the midge's salivary glands and eggs and can be excreted—without killing the midge in the process—brings Drolet closer to confirming that *Culicoides* is a vector of VSV. "These results indicate that there are three possible ways the midge could be transmitting the virus," she says.

She'll be conducting three types of transmission studies: Horizontal-transmission studies will test whether the midges spread VSV to animals through biting. Vertical-transmission studies will test whether the virus spreads from adult midges to their offspring. And mechanical-transmission studies will test whether virus excreted by infected midges can be passed to uninfected insects.

"If the midge transmits the virus in all three ways, then it's likely that it's a very efficient transmission vector for VSV in nature," says Drolet.—By **Rosalie Marion Bliss,** ARS.

This research is part of Veterinary, Medical, and Urban Entomology (#104) and Animal Health (#103), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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